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Article

Seasonal Variability in the Essential Oils of Wild and Cultivated *Baccharis trimera*

Fabiano G. Silva,[a] Carolina B. A. Oliveira,[b] José Eduardo B. P. Pinto,[c] Vivian E. Nascimento,[c] Suzana C. Santos,[b] José C. Seraphin[d] and Pedro H. Ferri*[,b]

[a]*Laboratório de Cultura de Tecidos, Centro Federal de Educação Tecnológica de Rio Verde, CP 66, 75900-000 Rio Verde-GO, Brazil*

[b]*Laboratório de Bioatividade Molecular, Instituto de Química, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia-GO, Brazil*

[c]*Laboratório de Cultura de Tecidos e Plantas Medicinais, Departamento de Agricultura, Universidade Federal de Lavras, CP 37, 37200-000 Lavras-MG, Brazil*

[d]*Núcleo de Estatística Aplicada, Instituto de Matemática e Estatística, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia-GO, Brazil*

A variação sazonal na composição química do óleo essencial em populações de *Baccharis trimera* natural e cultivada indicou a presença de três grupos de óleos em relação à origem e à fase de desenvolvimento das amostras. O primeiro incluiu amostras floridas, silvestres ou cultivadas, no período entre março-maio, contendo altas percentagens de globulol e espatulenol. No grupo II, com amostras silvestres coletadas entre junho-fevereiro, os constituintes majoritários foram o germacreno D e o (*E*)-cariofileno, enquanto que o grupo III incluiu amostras cultivadas entre junho-fevereiro e contendo um alto conteúdo de ledol. A análise por correlação canônica indicou que (*E*)-cariofileno, Mn, saturação por Al e precipitação foram fortemente correlacionados às amostras silvestres (grupo II), enquanto ledol e guaiol correlacionaram-se ao balanço químico do solo (P, K, S, Cu, Zn e saturação por bases) nas amostras cultivadas (grupo III). Ambos os óleos essenciais tem predominantemente hidrocarbonetos, embora em dois meses o conteúdo em sesquiterpenos oxigenados seja superior a 40% para ambas as amostras. As variações observadas podem estar relacionadas ao ambiente.

The seasonal variations in the chemical composition of the essential oil of wild and cultivated *Baccharis trimera* populations indicated the presence of three clusters of oils regarding population and phenophase. The first included flowering, wild, and cultivated samples from a period of March-May, with high percentages of globulol and spathulenol. In cluster II, with wild samples collected from June-February, the major constituents were germacrene D and (*E*)-caryophyllene, while cluster III included cultivated samples from June-February containing a high content of ledol. The canonical correlation analysis revealed that (*E*)-caryophyllene, Mn, Al saturation, and precipitation were quite strongly related to wild samples (cluster II), whereas ledol and guaiol were related to chemical balance in soils (P, K, S, Cu, Zn, and base saturation) of cultivated samples (cluster III). Both essential oils have predominantly hydrocarbon compositions, although for a couple of months the oxygenated sesquiterpene content is over 40% for both samples. The observed chemovariation might be environmentally determined.

Keywords: *Baccharis trimera*, Asteraceae, essential oil, chemical variability, seasonal variation

Introduction

Baccharis species (Asteraceae) are widespread in South America and are known as 'carqueja'. Several species are used in traditional medicine or are reputed as toxic to cattle and sheep.[1] Medicinal teas prepared from the flowering *B. trimera* (Less.) DC. (synon. *B. genistelloides* var. *trimera* (Less.) Baker) are used to treat gastrointestinal, renal and liver diseases, diabetes, rheumatism, and may act as anti-inflammatory.[2] Other

*e-mail: pedro@quimica.ufg.br

popular uses in Brazil show that *B. trimera* is employed in the treatment of malaria, sore throat and tonsillitis, angines, anaemia, urinary inflammation and leprosy.[2-4] Liver-protective properties, gastrointestinal action, potential antidiabetic activity, and anti-arthritic effects have been validated,[5,6] and the anti-ophidian, relaxant, antimutagenic, antimicrobial, and antiprotozoal activities have also been reported.[7,8]

Chemical studies revealed the presence of clerodane-type diterpenes, saponins and their glycosides, and several flavonoids in this species.[5,7] Carqueja oil, a commercial fragrance cited as a reminiscent of rosewood,[9] is stem-distilled from its aerial parts, with the unusual monoterpene alcohol carquejol and its acetyl ester as major components.[10-17] The commercial value of this oil which is produced in Brazil has been associated with the high content of carquejyl acetate which has showed variations of 30% to 69.2%.[3,9,14] Carquejyl acetate was found in *B. trimera* which was collected in different places of southern Brazil and Argentina, and has been regarded as a chemomarker of this species,[17] although it has been absent in collected samples in Paraná and Santa Catarina states, South Brazil,[18] and identified in other species of the same genera.[15]

Despite the great demand of *B. trimera* in the phytotherapeutic industry,[18,19] as well as the demand for fragrance - *B. trimera*'s essential oil is cited as one of the ten most consumed oils by cosmetic industries in Brazil[20] - the variability in essential oil of wild and cultivated plants has not yet been obtained.[21] In fact, natural populations have been excessively exploited and their natural habitats have been replaced by commercial crops. As a result, the amount of this wild plant has decreased.[21]

As part of our ongoing work on the characterization of essential oils of medicinal aromatic plants growing wild in the Brazilian Cerrado,[22] we now report on the results obtained for the essential oil composition and seasonal variability of *B. trimera* that was collected from wild and cultivated populations during a 1-year period. For this purpose, essential oils from aerial parts of representative population samples of each origin were analyzed by GC-MS. In order to study chemical variability, chemical constituents were submitted to Principal Component, chemometric Cluster, and Canonical Discriminant analysis in order to detect the pattern distribution of samples and to identify which constituents can distinguish between these groups of individuals. In addition, environmental factors affecting essential oil variability were studied through the application of a Canonical Correlation analysis between the oil component data set and the edaphic-climactic data matrix with 26 variables for each sampling.

Results and Discussion

B. trimera essential oils were obtained from wild and from cultivated populations. The annual mean yield of oils from wild samples was higher than that of the cultivated plants. Previous work has indicated that organic fertilizer and shade showed no significant effects on the essential oil yield,[21,23] but radiation increased the content of essential oils in cultivated carqueja.[24] The seasonal dynamics of essential oil yield in both populations were similar to those of other Cerrado species, which revealed lower values during the dry winter.[25] In total, 26 compounds were identified, accounting for 96-100% of the volatile constituents (Table 1). Both essential oils have predominantly sesquiterpene hydrocarbon compositions, although for a couple of months the oxygenated sesquiterpene content is over 40% for both samples. Essential oils from the *Baccharis* species have showed that, although the main constituent may vary, sesquiterpenes are usually the dominant class.[26] Furthermore, important differences in the amounts of the major constituents were found, mainly of germacrene D (6.3-28%) which had the highest percentage in September-November (both populations); ledol (2.5-16%) showed a high variation in the wild population, while cultivated samples revealed higher amounts, regardless of the months; and bicyclogermacrene (12-24%) had the highest contents between July-February in the wild population, and in December-February in cultivated ones.

The results obtained from PCA and nearest neighbour complete linkage cluster analysis using Ward's technique revealed the existence of a high chemical variability within the essential oils of *B. trimera*.[27] Figure 1 shows the relative position of the individuals in the discriminant space in relation to an axial system that was originated in the PCA. First PC accounts for *ca.* 47% of the total variance and separates ($p < 0.0001$) the oxygenated sesquiterpenes of flowering sampling (March-May) from vegetative sampling, regardless of the populations, while the Second PC distinguishes ($p < 0.0001$) the wild sampling from that of the cultivated population in the June-April period. Therefore, three types of essential oils were found: cluster I (wild and cultivated samples harvested in March-May), being characterised by a high percentage ($p < 0.0001$) of globulol (8.9 ± 3.4%), spathulenol (8.7 ± 3.1%), α-guaieno (3.4 ± 0.4%), viridiflorol (3.4 ± 0.9%), guaiol (3.1 ± 0.8%), and δ-cadinene (6.6 ± 1.9%) ($p < 0.007$); cluster II (wild samples harvested between June-February) with ($p < 0.0001$) germacrene D (26 ± 3%) and (*E*)-caryophyllene

Table 1. Mean values[a] of volatile components from populations of cultivated or nature-collected *B. trimera* during the year

Constituents	KI	Population	January	February	March	April	May	June	July	August	September	October	November	December
α-Copaene	1376	Cultivated	1.3 ^{Ba}	1.1 ^{Ba}	1.3 ^{Ba}	2.4 ^{Aa}	2.0 ^{Aa}	0.56 ^{Ca}	1.6 ^{Aa}	0.50 ^{Ca}	1.4 ^{Ba}	2.0 ^{Aa}	1.6 ^{Aa}	0.99 ^{Ba}
		Wild	1.7 ^{Aa}	1.3 ^{Aa}	1.2 ^{Aa}	1.4 ^{Ab}	1.7 ^{Aa}	1.2 ^{Aa}	0.97 ^{Aa}	0.93 ^{Aa}	1.3 ^{Aa}	1.5 ^{Aa}	1.7 ^{Aa}	1.6 ^{Aa}
β-Cubebene	1390	Cultivated	-	-	-	0.22 ^{A}	-	-	0.13 ^{Aa}	-	-	0.24 ^{Aa}	-	-
		Wild	0.06 ^{B}	-	-	-	-	-	0.26 ^{Aa}	0.01 ^{B}	0.13 ^{A}	0.25 ^{Aa}	0.14 ^{A}	-
β-Elemene	1392	Cultivated	0.77 ^{Ca}	0.36 ^{Ca}	0.01 ^{Ca}	0.99 ^{Ca}	0.14 ^{Ca}	0.86 ^{Ba}	0.62 ^{Ba}	0.60 ^{Ba}	0.81 ^{Ba}	1.2 ^{Aa}	0.37 ^{Cb}	0.57 ^{Ba}
		Wild	0.21 ^{Ca}	0.54 ^{Ba}	0.01 ^{Ca}	0.01 ^{Ca}	0.44 ^{Ba}	0.62 ^{Ba}	0.74 ^{Aa}	0.59 ^{Ba}	0.63 ^{Ba}	0.81 ^{Ab}	1.1 ^{Aa}	0.81 ^{Aa}
α-Gurjunene	1410	Cultivated	0.59 ^{Aa}	0.94 ^{Aa}	0.24 ^{Ba}	0.25 ^{Ba}	0.31 ^{Ba}	0.90 ^{Aa}	0.69 ^{Aa}	0.63 ^{Aa}	0.51 ^{Aa}	0.59 ^{Aa}	0.54 ^{Aa}	0.66 ^{Aa}
		Wild	0.24 ^{Ab}	0.18 ^{Ab}	0.15 ^{Aa}	0.11 ^{Aa}	0.23 ^{Aa}	0.47 ^{Ab}	0.20 ^{Ab}	0.31 ^{Aa}	0.37 ^{Ab}	0.11 ^{Ab}	-	0.25 ^{Ab}
(*E*)-Caryo-phyllene	1420	Cultivated	16 ^{Ab}	17 ^{Ab}	16 ^{Aa}	16 ^{Aa}	17 ^{Aa}	14 ^{Bb}	13 ^{Bb}	12 ^{Bb}	14 ^{Ba}	14 ^{Ba}	14 ^{Ba}	16 ^{Ab}
		Wild	21 ^{Aa}	20 ^{Aa}	16 ^{Ba}	16 ^{Ba}	19 ^{Aa}	19 ^{Aa}	17 ^{Ba}	17 ^{Ba}	17 ^{Ba}	16 ^{Ba}	17 ^{Ba}	20 ^{Aa}
α-Guaiene	1439	Cultivated	2.4 ^{Ba}	2.4 ^{Ba}	3.4 ^{Aa}	3.7 ^{Aa}	3.7 ^{Aa}	2.4 ^{Ba}	1.9 ^{Ca}	1.9 ^{Ca}	1.7 ^{Ca}	1.6 ^{Ca}	2.2 ^{Ba}	2.5 ^{Ba}
		Wild	2.6 ^{Ca}	3.0 ^{Ba}	3.3 ^{Aa}	3.8 ^{Aa}	2.8 ^{Bb}	2.5 ^{Ca}	1.9 ^{Ba}	2.0 ^{Ba}	1.9 ^{Da}	2.1 ^{Da}	2.4 ^{Ca}	2.9 ^{Ba}
α-Humulene	1454	Cultivated	1.4 ^{Bb}	1.4 ^{Bb}	1.8 ^{Aa}	1.9 ^{Aa}	1.6 ^{Ab}	1.3 ^{Bb}	1.4 ^{Ba}	1.2 ^{Bb}	1.4 ^{Ba}	1.6 ^{Bb}	1.2 ^{Bb}	1.4 ^{Bb}
		Wild	1.9 ^{Aa}	1.8 ^{Ba}	2.0 ^{Aa}	1.8 ^{Ba}	1.9 ^{Aa}	1.7 ^{Ba}	1.6 ^{Ba}	1.6 ^{Ba}	1.6 ^{Ba}	1.9 ^{Aa}	1.8 ^{Ba}	2.1 ^{Aa}
γ-Gurjunene	1472	Cultivated	0.55 ^{Aa}	0.60 ^{Aa}	0.27 ^{Aa}	0.11 ^{A}	0.94 ^{Aa}	0.86 ^{Aa}	0.69 ^{A}	0.58 ^{Aa}	0.37 ^{A}	0.54 ^{A}	1.1 ^{Aa}	0.73 ^{Aa}
		Wild	0.08 ^{Aa}	0.07 ^{Aa}	0.10 ^{Aa}	-	0.92 ^{Aa}	0.30 ^{Aa}	-	0.16 ^{Aa}	-	-	0.46 ^{Aa}	0.27 ^{Aa}
γ-Muurolene	1477	Cultivated	1.6 ^{Ca}	1.4 ^{Ca}	2.1 ^{Ca}	3.8 ^{Aa}	2.5 ^{Ba}	1.1 ^{Ca}	1.2 ^{Ca}	0.9 ^{Ca}	1.3 ^{Ca}	1.2 ^{Ca}	1.6 ^{Ca}	1.4 ^{Cb}
		Wild	2.2 ^{Ba}	2.0 ^{Ba}	2.0 ^{Ba}	3.0 ^{Ab}	2.6 ^{Aa}	1.4 ^{Ca}	0.97 ^{Ca}	1.0 ^{Ca}	1.2 ^{Ca}	1.2 ^{Ca}	1.4 ^{Ca}	2.1 ^{Ba}
Germacrene D	1483	Cultivated	25 ^{Aa}	25 ^{Aa}	7.7 ^{Da}	12 ^{Ca}	6.3 ^{Db}	18 ^{Ba}	22 ^{Ab}	20 ^{Bb}	25 ^{Aa}	26 ^{Aa}	23 ^{Aa}	20 ^{Ba}
		Wild	24 ^{Ba}	27 ^{Aa}	8.9 ^{Ca}	12 ^{Ca}	13 ^{Ca}	20 ^{Ba}	27 ^{Aa}	27 ^{Aa}	28 ^{Aa}	28 ^{Aa}	26 ^{Aa}	23 ^{Ba}
β-Selinene	1487	Cultivated	0.75 ^{Ba}	0.74 ^{Ba}	1.2 ^{Aa}	1.3 ^{Aa}	1.1 ^{Aa}	0.62 ^{Ba}	-	0.52 ^{Ba}	0.57 ^{Ba}	0.41 ^{Ba}	0.90 ^{Aa}	0.93 ^{Aa}
		Wild	0.79 ^{Ba}	0.77 ^{Ba}	1.3 ^{Aa}	1.1 ^{Aa}	0.86 ^{Ba}	0.85 ^{Ba}	0.41 ^{C}	0.49 ^{Ca}	0.60 ^{Ca}	0.67 ^{Ca}	0.76 ^{Ba}	0.91 ^{Ba}
Bicyclo-germacrene	1497	Cultivated	23 ^{Aa}	23 ^{Aa}	14 ^{Ba}	14 ^{Ba}	12 ^{Ba}	20 ^{Aa}	17 ^{Bb}	24 ^{Aa}	16 ^{Bb}	15 ^{Bb}	15 ^{Bb}	21 ^{Aa}
		Wild	20 ^{Aa}	22 ^{Aa}	15 ^{Ba}	15 ^{Ba}	13 ^{Ba}	17 ^{Ba}	23 ^{Aa}	23 ^{Aa}	21 ^{Aa}	22 ^{Aa}	20 ^{Aa}	19 ^{Aa}
α-Muurulene	1500	Cultivated	1.0 ^{Ba}	0.88 ^{Ba}	1.1 ^{Ba}	2.1 ^{Aa}	1.7 ^{Aa}	0.48 ^{Bb}	0.80 ^{Ba}	0.45 ^{Ba}	0.78 ^{Ba}	0.86 ^{Ba}	0.82 ^{Ba}	0.90 ^{Ba}
		Wild	1.3 ^{Aa}	1.1 ^{Ba}	1.0 ^{Ba}	1.5 ^{Ab}	1.5 ^{Aa}	0.92 ^{Ba}	0.52 ^{Ba}	0.68 ^{Ba}	0.88 ^{Ba}	0.71 ^{Ba}	1.1 ^{Ba}	1.1 ^{Ba}
α-Bulnecene	1506	Cultivated	1.0 ^{Aa}	1.0 ^{Aa}	0.43 ^{Ba}	0.34 ^{Ba}	0.10 ^{Bb}	1.2 ^{Aa}	0.92 ^{Aa}	1.2 ^{Aa}	0.99 ^{Aa}	1.1 ^{Aa}	0.73 ^{Ab}	0.86 ^{Aa}
		Wild	1.1 ^{Aa}	1.1 ^{Aa}	0.39 ^{Ba}	0.46 ^{Ba}	0.45 ^{Ba}	1.2 ^{Aa}	0.95 ^{Aa}	1.3 ^{Aa}	1.2 ^{Aa}	1.3 ^{Aa}	1.1 ^{Aa}	0.92 ^{Aa}
γ-Cadinene	1515	Cultivated	0.48 ^{Aa}	0.28 ^{Ba}	0.76 ^{Aa}	1.3 ^{Aa}	1.0 ^{Aa}	-	0.72 ^{A}	-	-	0.08 ^{Ba}	0.31 ^{Ba}	0.43 ^{Ba}
		Wild	0.85 ^{Aa}	0.77 ^{Aa}	0.77 ^{Aa}	1.3 ^{Aa}	0.87 ^{Aa}	0.14 ^{B}	-	0.05 ^{B}	0.25 ^{B}	0.07 ^{Ba}	0.54 ^{Aa}	0.64 ^{Aa}
δ-Cadinene	1523	Cultivated	5.5 ^{Aa}	4.2 ^{Ba}	5.8 ^{Aa}	8.7 ^{Aa}	5.9 ^{Aa}	3.8 ^{Ba}	5.1 ^{Ba}	3.0 ^{Ba}	6.4 ^{Aa}	6.1 ^{Aa}	6.2 ^{Aa}	4.7 ^{Bb}
		Wild	6.2 ^{Aa}	6.3 ^{Aa}	6.1 ^{Aa}	5.2 ^{Ab}	6.8 ^{Aa}	5.1 ^{Aa}	5.0 ^{Aa}	4.5 ^{Aa}	6.0 ^{Aa}	6.4 ^{Aa}	7.4 ^{Aa}	7.3 ^{Aa}
δ-Calacorene	1544	Cultivated	-	-	-	-	-	-	-	-	-	-	-	-
		Wild	-	-	0.16	-	-	-	-	-	-	-	-	-
Germacrene B	1558	Cultivated	0.23 ^{Ba}	0.18 ^{Ba}	-	-	0.15 ^{Ba}	0.06 ^{Ba}	2.2 ^{Aa}	0.28 ^{Ba}	0.06 ^{Ba}	-	0.18 ^{Ba}	0.05 ^{Ba}
		Wild	0.30 ^{Aa}	0.36 ^{Aa}	-	1.2 ^{A}	0.31 ^{Aa}	0.27 ^{Aa}	0.16 ^{Ab}	0.64 ^{Aa}	0.36 ^{Aa}	0.28 ^{A}	0.53 ^{Aa}	0.52 ^{Aa}
Ledol	1570	Cultivated	7.5 ^{Ba}	10 ^{Aa}	12 ^{Aa}	4.2 ^{Ba}	9.7 ^{Aa}	16 ^{Aa}	12 ^{Aa}	13 ^{Aa}	11 ^{Aa}	12 ^{Aa}	9.6 ^{Aa}	12 ^{Aa}
		Wild	2.9 ^{Ba}	2.8 ^{Ba}	9.6 ^{Aa}	3.6 ^{Ba}	9.3 ^{Aa}	8.6 ^{Ab}	5.9 ^{Bb}	7.2 ^{Ab}	4.8 ^{Bb}	6.1 ^{Bb}	4.4 ^{Bb}	4.1 ^{Bb}
Spathulenol	1578	Cultivated	2.6 ^{Da}	1.8 ^{Da}	12 ^{Aa}	5.2 ^{Ca}	9.4 ^{Ba}	6.6 ^{Ca}	6.4 ^{Ca}	6.3 ^{Ca}	5.1 ^{Ca}	3.1 ^{Da}	3.0 ^{Da}	4.0 ^{Da}
		Wild	3.0 ^{Ca}	1.2 ^{Ca}	12 ^{Aa}	6.6 ^{Ba}	7.1 ^{Bb}	6.4 ^{Ba}	3.5 ^{Cb}	3.7 ^{Cb}	3.3 ^{Ca}	2.1 ^{Ca}	2.5 ^{Ca}	3.1 ^{Ca}
Globulol	1583	Cultivated	3.8 ^{Da}	3.1 ^{Da}	8.3 ^{Ba}	8.6 ^{Ba}	11 ^{Aa}	4.8 ^{Ca}	5.8 ^{Ca}	6.2 ^{Ca}	6.2 ^{Ca}	5.0 ^{Ca}	3.0 ^{Da}	4.7 ^{Ca}
		Wild	4.2 ^{Ba}	2.9 ^{Ba}	8.8 ^{Aa}	8.9 ^{Aa}	6.9 ^{Ab}	5.1 ^{Ba}	4.0 ^{Ba}	3.8 ^{Ba}	4.3 ^{Ba}	3.7 ^{Ba}	3.6 ^{Ba}	3.9 ^{Ba}
Viridiflorol	1592	Cultivated	1.4 ^{Ba}	1.3 ^{Ba}	3.5 ^{Aa}	3.5 ^{Aa}	3.6 ^{Aa}	1.5 ^{Ba}	1.5 ^{Ba}	1.5 ^{Ba}	0.74 ^{Ca}	0.54 ^{Ca}	0.67 ^{Ca}	1.7 ^{Ba}
		Wild	2.0 ^{Ca}	1.6 ^{Ca}	2.9 ^{Ba}	4.3 ^{Aa}	2.6 ^{Bb}	1.4 ^{Ca}	1.3 ^{Ca}	1.1 ^{Ca}	1.5 ^{Ca}	1.2 ^{Ca}	1.4 ^{Ca}	1.7 ^{Ca}
Guaiol	1604	Cultivated	2.2 ^{Ba}	2.5 ^{Ba}	3.5 ^{Aa}	3.5 ^{Ba}	3.8 ^{Aa}	3.5 ^{Aa}	1.9 ^{Ba}	2.8 ^{Ba}	2.4 ^{Ba}	2.6 ^{Ba}	2.7 ^{Ba}	3.0 ^{Ba}
		Wild	0.90 ^{Bb}	0.88 ^{Bb}	3.3 ^{Aa}	3.0 ^{Aa}	2.7 ^{Aa}	1.7 ^{Bb}	1.4 ^{Ba}	1.5 ^{Bb}	1.2 ^{Bb}	0.77 ^{Bb}	1.5 ^{Ba}	1.0 ^{Bb}
epi-α-Cadinol	1640	Cultivated	-	-	1.0 ^{Aa}	0.30 ^{Ab}	0.48 ^{Aa}	0.45 ^{Aa}	0.44 ^{Aa}	0.29 ^{Aa}	0.16 ^{Aa}	0.06 ^{A}	-	0.42 ^{Aa}
		Wild	-	0.41 ^{B}	0.94 ^{Aa}	1.5 ^{Aa}	0.08 ^{Ba}	0.33 ^{Ba}	0.34 ^{Ba}	0.18 ^{Ba}	0.06 ^{Ba}	-	0.27 ^{B}	0.26 ^{Ba}
α-Cadinol	1655	Cultivated	1.1 ^{Ba}	1.0 ^{Ba}	2.4 ^{Aa}	2.9 ^{Ab}	2.7 ^{Aa}	0.99 ^{Ba}	0.76 ^{Ba}	0.84 ^{Ba}	1.2 ^{Ba}	0.95 ^{Ba}	0.99 ^{Ba}	1.2 ^{Ba}
		Wild	1.6 ^{Ba}	1.4 ^{Ba}	2.5 ^{Ba}	4.7 ^{Aa}	2.3 ^{Ba}	1.1 ^{Ba}	1.3 ^{Ba}	0.90 ^{Ba}	1.0 ^{Ba}	1.5 ^{Ba}	1.7 ^{Ba}	1.4 ^{Ba}
Eudesma-4(15),7-dien-1-β-ol	1691	Cultivated	0.37 ^{Ba}	0.32 ^{Ba}	0.23 ^{Ba}	0.29 ^{Ba}	-	-	0.08 ^{B}	0.12 ^{Ba}	0.63 ^{Aa}	0.81 ^{Aa}	0.66 ^{Aa}	0.07 ^{Ba}
		Wild	0.15 ^{Ba}	0.12 ^{Ba}	-	0.31 ^{Aa}	0.08 ^{B}	-	-	0.07 ^{Ba}	0.54 ^{Aa}	0.52 ^{Aa}	0.52 ^{Aa}	0.06 ^{Aa}
Sesquiterpene hydrocarbons		Cultivated	81 ^{Aa}	79 ^{Ab}	56 ^{Ca}	68 ^{Ba}	56 ^{Cb}	65 ^{Ba}	68 ^{Bb}	68 ^{Bb}	72 ^{Bb}	72 ^{Bb}	77 ^{Aa}	73 ^{Bb}
		Wild	84 ^{Aa}	88 ^{Aa}	58 ^{Ca}	69 ^{Ba}	66 ^{Ba}	72 ^{Ba}	81 ^{Aa}	80 ^{Aa}	83 ^{Aa}	84 ^{Aa}	83 ^{Aa}	84 ^{Aa}
Oxygenated sesquiterpenes		Cultivated	19 ^{Da}	20 ^{Da}	42 ^{Aa}	27 ^{Ca}	41 ^{Aa}	34 ^{Ba}	31 ^{Ca}	31 ^{Ca}	28 ^{Ca}	25 ^{Ca}	25 ^{Ca}	27 ^{Ca}
		Wild	15 ^{Ca}	11 ^{Cb}	40 ^{Aa}	28 ^{Ba}	32 ^{Bb}	25 ^{Bb}	18 ^{Cb}	19 ^{Cb}	17 ^{Cb}	16 ^{Cb}	16 ^{Cb}	16 ^{Cb}
Oil yield/(%)		Cultivated	0.25 ^{Aa}	0.27 ^{Ab}	0.22 ^{Ba}	0.23 ^{Ba}	0.19 ^{Ca}	0.17 ^{Ca}	0.13 ^{Db}	0.12 ^{Da}	0.12 ^{Da}	0.11 ^{Da}	0.16 ^{Ca}	0.15 ^{Cb}
		Wild	0.28 ^{Ba}	0.32 ^{Aa}	0.23 ^{Ca}	0.23 ^{Ca}	0.17 ^{Da}	0.16 ^{Da}	0.17 ^{Da}	0.14 ^{Ea}	0.13 ^{Ea}	0.12 ^{Ea}	0.18 ^{Da}	0.22 ^{Ca}

[a] Percentage data. Means followed by the same capital letter in the rows and same small letter in the columns did not share significant differences at 5% probability by Scott-Knott test. (-): not detected.

(19 ± 2%) as principal constituents; and cluster III (cultivated samples harvested in the June-February period) containing a high amount of ledol (12 ± 3%) ($p < 0.0001$).

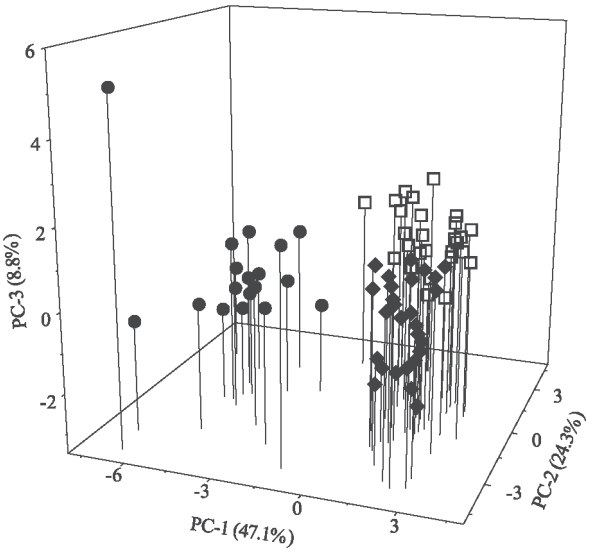

Figure 1. Principal Component scatterplot of *B. trimera* samples collected from wild and cultivated populations to which cluster it belongs: I (●), II (□) and III (◆).

The canonical discriminant analysis (CDA) confirmed this clustering as *a priori* groupings and a two-dimensional axial system that originated in the CDA distinguished the different types of essential oils based on the contents of globulol, germacrene D and ledol as predictor variables (Figure 2). The first discriminant function (F1) accounts for *ca.* 90% of the total variability and separates the samples in flowering stage ($p < 0.0001$), regardless of the population, due to the high content of globulol. On the other hand, the second discriminant function (F2) distinguishes the harvested samples according to the wild or cultivated population ($p < 0.0003$), as a result of the high contents of germacrene D and ledol, respectively. In addition, when using the two discriminant functions it is possible to predict accurately *ca.* 96% well classification in the original clusters (only one misclassification) by a cross-validation approach. This approach involves taking a slightly reduced number of samples from the parent data set, estimating parameters from each of these modified data sets and then calculating the precision of the predictions for the samples previously removed by the resulting models.[28] The only observed misclassification was the cultivated sample harvested in January (Figure 2), which was classified as belonging to the wild population. Such misclassification could be caused by lower levels of ledol during January (a characteristic of wild samples).

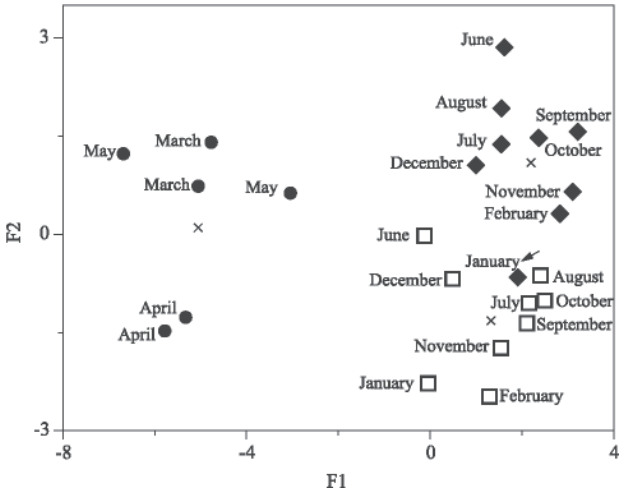

Figure 2. Canonical discriminant-scatterplot of *B. trimera* sampling months from wild and cultivated populations to which cluster it belongs: I (●), II (□) and III (◆). Arrow shows the misclassification individual. Crosses represent the group centroids on the canonical variates.

All these findings may be correlated with factors other than genetic determination as biotic pressures which could modulate the volatiles of cluster I (flowering phase) from clusters II and III (vegetative phase), the former being influenced by pollinators and the latter by pathogens and herbivores or because of differences in environmental conditions.[29] In fact, the canonical correlation analysis (Table 2) between populations and soils revealed that ledol and guaiol present a strong relationship with chemical balance in soils (P, K, S, Cu, Zn, and base saturation) and are related to the cultivated samples collected in the June-February period (cluster III). In addition, (*E*)-caryophyllene from the first set and Mn, Al saturation, soil humidity, and precipitation from the second set load fairly strongly onto the first canonical variable, which is related to the wild samples collected in the June-February period (cluster II).

Unlike previous studies which used plants that were collected in different places of southern Brazil and Argentina,[10-17] where carquejol reached 0-9% and carquejyl acetate 12.2-69.2%, our results did not reveal any of these chemicals. By contrast, germacrene D (21 ± 7%), bicyclogermacrene (19 ± 4%), (*E*)-caryophyllene (17 ± 3%), ledol (8.3 ± 4.0%), δ-cadinene (5.8 ± 1.6%), globulol (5.4 ± 2.5%), and spathulenol (5.0 ± 3.1%) were always present as major compounds in our samples, whereas in previous studies some of these chemicals were obtained as minor constituents or were only reported in trace amounts.[11-13,16,17] Some studies report high contents of ledol (9.93 ± 7.34%),[16,17] whereas other researches did not find these volatiles at all.[10,14,15] The lack of carquejol and carquejyl acetate was also reported in samples

Table 2. Canonical correlation structure (loadings) of the oil components and edapho-climactic factors with their canonical variates

Discriminant oil constituents (set 1)	Canonical variate V1	Edapho-climactic factors (set 2)	Canonical variate W1
(*E*)-Caryophyllene	–0.7452	Clay / (%)	0.8807
α-Guaiene	–0.3039	P / (mg dm^{-3})	0.8807
Germacrene D	–0.1161	K / (mg dm^{-3})	0.8807
Bicyclogermacrene	–0.1248	S / (mg dm^{-3})	0.8807
δ-Cadinene	–0.2408	Cu / (mg dm^{-3})	0.8807
Ledol	0.8503	Mn / (mg dm^{-3})	–0.8807
Spathulenol	0.2336	Zn / (mg dm^{-3})	0.8807
Globulol	0.2303	Base saturation / (%)	0.8807
Guaiol	0.6321	Al saturation / (%)	–0.8807
		Temperature / (°C)	–0.2885
		Precipitation / (mm)	–0.3745
		Soil humidity / (%)	–0.3114
Eingenvalue			0.6555
Canonical correlation			0.8097
Wilks' lambda			0.0833
Bartlett's *Chi*-square			151.62
Degrees of freedom			108
P-value			0.0036
Cumulative variance / (%):			
of discriminant oil components data			14.31
of discriminant oil components-edapho-climactic relation			39.89

collected in Santa Catarina and Paraná states in South Brazil, in which essential oils were obtained by different methods, such as hydrodistillation by Clevenger apparatus, supercritical fluid extraction, and hexane extract.[18]

To judge from the current available data, a trend of oxidised monoterpenes seems to accompany the transition of sampling sites from the Brazilian Cerrado (present study) to sites in southern Brazil and Argentina. The same pattern is found when the climate is considered. Along this direction, a gradual change from the hot and dry to the cold and humid Austral Continental type occurs,[30] suggesting that edapho-climactic variations may explain polymorphism in essential oils. A similar oxidative gradient was described for the emmotin sesquiterpenoids in South America,[31] and for monoterpenes and sesquiterpenes in *Hyptis suaveolens* (Lamiaceae), but with an increase in the oxidised level from the Brazilian Cerrado to the Amazonian region.[32]

Thus, the variation pattern in the essential oil may reflect selective pressures in the different ecological and geographical environments (ecotypes) or indicate that the observed chemical variations could be due to the existence of chemotypes for *B. trimera*, which have not yet been described for *Baccharis* species. Therefore, caution is necessary in the use of carquejyl acetate as a chemomarker of *B. trimera* for this compound could be in part environmentally determined. In spite of the correlation obtained for the oil composition with edapho-climactic factors and the variance explained by the environmental data set (Table 2), there is an outstanding percentage of variability in oil composition that should be the subject of subsequent genetic studies.

Experimental

Plant material

B. trimera samples were collected between March 2003 and February 2004 in Lavras city (S 21° 13'; W 44° 57'; 919 m), Minas Gerais State, Brazil, and were identified by Prof. Dr. Manoel Losada Gavilanes of Departamento de Biologia, Universidade Federal de Lavras (UFLA), Minas Gerais State, Brazil. Samples from their natural habitat were collected on a monthly basis, and plantlets obtained by cutting propagation were cultivated in an experimental field at the Setor de Horticultura of Universidade Federal de Lavras (UFLA),[21] Minas Gerais State, Brazil. The natural habitat was located at 20 km from the cultivated samples. Field trial was performed in the form of a randomised block with three replications. In the March-May period, the plants included both leaves and flowers. Voucher specimen is deposited in UFLA's Herbarium (code number 169933).

In order to assess the chemical composition of oils, the aerial parts of 40-100 randomised individual plants - representing the local population as homogeneous samples

- were simultaneously collected from cultivated plants and from their original natural habitat and dried by dehumidification in Arsec 160 apparatus for 3 days at 35 °C until constant weight. After having been chopped, the dried phytomass (50 g) was submitted to hydro-distillation (1 h) using a modified Clevenger-type apparatus. At the end of each distillation, oils were collected by extracting the aqueous solution with CH_2Cl_2 (3 × 90 mL), drying the dichloromethane layer with anhydrous $MgSO_4$, and removing the solvent in a rotavapor under reduced pressure at 40 °C. The oils were transferred to glass flasks and kept at a temperature of –18 °C. Oil yields (%) were based on the dried weight of plant samples. All experiments were conducted in triplicates.

Soil analyses

Five-soil samples were collected at a 20 cm depth in each locality in March 2003. These samples were then pooled together to form one composite sample and subsequently air-dried, thoroughly mixed, and sieved (2 mm). The portion which was finer than 2 mm was kept for physical and chemical analysis.[33] The pH was determined in a 1:1 soil-water volume ratio. Ca, Mg and Al were extracted with 1mol L^{-1} KCl, and P, K, Zn, Cu, Fe, Mn were extracted with Mehlich's solution. Organic matter, cationic exchange capacity (CEC), potential acidity (H+Al), base saturation, Al saturation, and soil texture were determined by applying the usual methods.[33] Soil humidity (at a 20 cm depth) was determined monthly by the gravimetric method, and was conducted in triplicates. Mean monthly values of temperature (minimal, average and maximal), precipitation, and humidity were obtained from UFLA's climatological station. Environmental factor data originating from both climatological records and soil analysis were ordered in an edapho-climactic matrix with 26 variables for each sampling site. In both data sets, essential oil components and edapho-climactic characteristics, a canonical correlation procedure was applied. This method finds the linear combinations of the variables which have the highest correlation between them.

In geographical terms, the natural habitat presents a loam texture, while the cultivated field has a sandy loam. Site elevation, mean annual rainfall, mean temperature, and mean annual relative humidity are, however, similar.

Chemical analysis

Oil sample analyses were performed on a GC-MS Shimadzu QP5050A instrument under the following conditions: a CBP-5 (Shimadzu) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) connected to a quadrupole detector operating in the EI mode at 70 eV with a scan mass range of 40-400 *m/z* at a sampling rate of 1.0 scan s^{-1}; carrier gas: He (1 mL min^{-1}); injector and interface temperatures of 220 °C and 240 °C, respectively, with a split ratio of 1:20. The injection volume was 0.2 μL (20% in CH_2Cl_2) and the oven temperature was raised from 60 °C to 246 °C, with an increase of 3 °C min^{-1}, then 10 °C min^{-1} to 270 °C, holding the final temperature for 5 min. Individual components were identified by comparing their Kovats retention indexes (RI),[34] made by a co-injection with a C_8-C_{32} *n*-alkanes series,[35] mass spectra with those of the literature, and a computerised MS-data base using NIST libraries.[34,36]

Chemical variability

Principal Component Analysis (PCA) was applied in order to examine the interrelationships between populations and their essential oil constituents using Système Portable d'Analyse des Données Numériques-SPAD.N software package, version 2.5, Centre International de Statistique et d'Informatique Appliquées, France (1994). Cluster analysis was also applied to the study of similarity of samples on the basis of essential oil constituent distribution. Nearest neighbour complete linkage technique by Benzécri algorithm was used as an index of similarity, and hierarchical clustering was performed according to the Ward´s variance minimizing method.[27,37]

Canonical discriminant analysis using SAS CANDISC procedure (Statistical Analysis System, SAS Institute Inc., Cary, NC, 1996) was used to differentiate between populations and clusters on the basis of oil composition. The predictive ability of canonical discriminant functions was evaluated by cross-validation leaving one group approach as implemented in SAS statistical package.

Oil variability and edapho-climactic factors relationships were obtained by a canonical correlation analysis implemented using the SAS CANCORR procedure. The predictive ability was evaluated by canonical redundancy analysis with a standardised variance coefficient.

Average multiple comparisons were established by the Scott-Knott test by ANOVA. *P*-values less than 0.05 were considered to be significant.

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Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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